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Organic osmolytes in human and other mammalian kidneys

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Organic osmolytes in human and other mammalian kidneys. Osmotically-active organic solutes, or osmolytes, have been found in high concentration in the renal inner medulla of a wide variety of mammalian species, but their existence in human kidneys has not yet been shown. The aim of this study was to demonstrate the presence of osmolytes in the human kidney. Human tissues were obtained from kidneys removed surgically for diseases which involved only one pole of the kidney; in most cases this was a tumor. Animal kidneys analyzed were from dogs, pigs and rabbits. Inner medulla and cortex tissue samples were analyzed and found to contain the organic osmolytes glycine betaine, myo-inositol, sorbitol and glycerophosphorylcholine. The levels were much higher in the medulla than in the cortex. Further dissection of the human kidneys showed that sorbitol, glycerophosphorylcholine and glycine betaine were maximally concentrated at the papillary tip, while myo-inositol was found in highest concentration at the papillary base. Osmolytes were in low concentrations or undetectable in rabbit skeletal muscle, ureter and bladder. The organic osmolytes detected are likely to be physiologically important in humans. Studies in other mammals can be used as models for the investigation of the osmolyte system in human kidney function.

The concentrating mechanism of the mammalian kidney creates an osmotic gradient in the extracellular fluid of the inner medulla. The osmotic pressure is maximal at the papillary tip, the major solutes being sodium, chloride and urea [1]. Sodium chloride is almost entirely in the extracellular space, whereas urea freely diffuses into cells [2]. The osmotic gradient presents two problems for the cells of the inner medulla. Firstly, as cells can not sustain large osmotic pressure differences across their membranes they must maintain comparable intra- and extracellular solute concentrations [3]. Secondly, the cells must maintain proteins in a functional form in the presence of high urea concentrations which would normally adversely affect protein structure and function [3, 4]. Recent studies have suggested that both of these problems are addressed by a common adaptive pathway.

The mechanism of adaptation in the inner medulla depends on the accumulation of high concentrations of certain low molecular weight organic substances, called "osmoprotectants" or osmolytes [5]. These maintain an osmotic balance without disturbing cellular function, and have therefore also been called "compatible" or "nonperturbing" solutes. The osmolytes may specifically counteract the disruptive effect of urea by maintaining the tertiary structure of macromolecules [3, 4].

In the kidney, the non-urea organic osmolytes which have been found in the inner medulla of animal species are glycine betaine, sorbitol (D-glucitol), glycerophosphorylcholine (GPC), and myo-inositol [6–11]. Their papillary concentrations have been shown to be higher in antidiuresis and decrease under diuretic conditions [11]. Sorbitol and myo-inositol are known components of human urine [12], and we have found that glycine betaine is also present [13]. However, no studies have been previously published on human kidneys. We report evidence that these same solutes are concentrated in the renal medulla of human and other mammalian species in a manner consistent with a general mammalian osmoprotectant strategy.

Methods

Tissues

The tissues were obtained from adult male New Zealand White rabbits, impounded stray dogs, pigs being slaughtered for meat, and surgical samples of human material. The dogs and pigs were allowed free access to food and water. The rabbits were kept in metabolic cages, allowed free access to food and divided into two groups, antidiuretic and diuretic. For diuresis water with 5% sucrose was provided for five days prior to sacrifice, while for antidiuresis no fluid was allowed for 72 hours prior to sacrifice. The dogs were killed by injection with a lethal dose of barbiturate, pigs by electrical stunning and exsanguination and the rabbits by decapitation. Kidney and other tissues were removed immediately. The kidneys were cut into transverse slices and wrapped in cling film. Tissue samples were placed into dry ice and immediately transferred to a −70°C freezer. The second kidney from each dog was stored at room temperature for two hours before being frozen at −70°C.

Human kidneys were shown to be diseased at only one pole by radiographic techniques including ultrasonography, intravenous urography and computerized tomography. The kidneys were removed surgically and packed in ice. The pole of the kidney which was unaffected by disease was sliced off and stored at −70°C. The indications for nephrectomy were: renal cell carcinoma ($N = 9$), transitional cell carcinoma ($N = 1$) and recurrent hemorrhage into a cyst ($N = 1$). The affected pole did not extend to the kidney region studied. One patient had diabetes mellitus with no evidence of diabetic nephropathy. No other patient was known to have other diseases affecting the

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kidney. The kidneys came from eight males and three females, with a median age of 64 years (range 40 to 84). Plasma creatinine concentrations were within the normal range in all 11 patients (0.06 to 0.13 mmol/liter). All patients received premedication before surgery. Seven were given a benzodiazopine (temazepam $N = 4$, triazolam $N = 2$, and nitrazepam $N = 1$), four papaveretum, two promethazine and one hyoscine. Drugs administered during anesthesia were: nitrous oxide ($N = 11$), fentanyl and thiopentone ($N = 9$), atracuronium ($N = 7$), isoflurane ($N = 6$), halothane ($N = 5$), vercuronium ($N = 5$) and glycomalonate ($N = 3$); propofol, droperidol and edrophonium were also used.

Extraction

Frozen segments of the kidneys were dissected. Segments of renal tissue outside the outer medullary stripe were regarded as cortex. The inner medulla including the papillary tip was removed by dissecting it from the outer medulla. Additional dissection of the human kidneys occurred along the cortico-papillary axis, with cortex, outer and inner stripes of outer medulla, papillary base and papillary tip samples obtained. The human and rabbit samples were weighed and homogenized in 10 vol/wt acetonitrile/methanol mixture (9:1). The homogenates were centrifuged (2000 g for 5 min) and the resultant supernatant removed. The pellet was resuspended in 10 vol/wt methanol/water (8:2) and vortexed for one minute before centrifugation (2000 g for 5 min) and removal of the second supernatant. Supernatants were stored at −30°C. The canine and porcine tissues were weighed and subsequently homogenized in ice-cold perchloric acid (10 vol/wt) as previously described [14].

Betaine assay

Betaines were measured by high performance liquid chromatography (HPLC) after derivatization, using the method we have previously published [15].

Briefly, for the human and rabbit samples the acetonitrile/methanol extracts were dried with anhydrous disodium hydrogen phosphate containing argentous oxide (9:1 wt/wt). The resultant mixture was centrifuged (1000 g for 2 min) and 200 μl of the supernatant added to 20 μl of a 10% suspension of magnesium oxide. After vortex mixing 50 μl of the derivatizing agent (100 mmol/liter 4-bromophenacyl triflate in acetonitrile) was added and mixing continued. The suspended magnesium oxide was removed by centrifugation (1000 g for 2 min), and the supernatant analyzed by HPLC.

For the dog and pig samples the perchloric acid extracts were desalted by ion-exchange using Dowex 50 cation exchange resin (H^+ form). Betaines were retained when the extract was passed through a mini-column (bed volume 0.5 to 2 times volume of extract) of resin. After washing the columns with deionized water, the betaines were preferentially eluted using excess 2 mol/liter ammonia solution. The eluates were freeze-dried and reconstituted with methanol. Portions (20 μl) of the methanol extract were evaporated to dryness under nitrogen at 80°C. Diisopropylethylamine (DIPEA) was added (100 μl of 2 mmol/liter in acetonitrile) to each residue and mixed, followed by 4′-bromo-2-hydroxyacetophenone trifluoromethane-sulfonate (bromophenacyl triflate: 100 μl of 10 mmol/liter in acetonitrile). After mixing, the extracts were allowed to stand for 10 minutes to ensure complete derivatization. Deionized water (100 μl) was then added to stop the reaction and destroy excess derivatizing agent. The mixture was centrifuged (1000 g for 2 min) and the supernatant analyzed by HPLC.

HPLC separation was carried out on Brownlee 13 cm ODS reverse phase columns (3 cm guard + 10 cm analytical) at 50°C with the mobile phase 85% acetonitrile and 15% triethylamine citrate buffer (17 mmol/liter, pH 6.3) containing 1.3 mmol/liter sodium lauryl sulfate. The betaine derivatives were detected by their absorbance at 260 nm.

Polyol assay

Polyols and GPC were separated by HPLC on sulfonated resin sugar columns after desalting.

The perchloric acid or combined acetonitrile/methanol and methanol/water extracts were passed successively through mini-columns of Dowex 50 (ammonium form) and BioRad AG1 (bicarbonate form) resins. The eluates were freeze-dried and reconstituted to their original volumes.

HPLC separation was carried out at 85°C in a series of Brownlee Polypore resin columns, with the eluting solvent deionized water (0.5 ml/min). The column sequence used was: (1) a 3 cm Pb^{2+} guard, (2) a 10 cm Pb^{2+} column, and (3) a 3 cm H^+ guard.

The detector was a segmented stream continuous flow analyzer made up of standard Technicon AA2 modules. The eluate was passed into a segmented stream of alkaline potassium permanganate (2.5 mmol/liter $KMnO_4$ and 2.5 mol/liter NaOH with 0.02% sodium dodecylbenzenesulfonate as wetting agent). This was passed through a heating coil at 75°C to a colorimeter (420 nm). The telemetry output of the colorimeter was monitored by the integration system.

Tissue urea and sodium

The two tissue supernatants from each of the rabbit and human kidneys were combined (1 vol acetonitrile/methanol:1 vol methanol/water) and analyzed for sodium using flame photometry. Solutions were 1:20 with 15 mmol/liter $LiNO_3$ and standards were treated identically.

The combined tissue supernatants were dried under nitrogen at 70°C, reconstituted with water and urea content measured by automated chemistry (Hitachi autoanalyzer model no. 717).

Statistical methods

All results are expressed as mean ± SEM, with N referring to the number of kidneys in a given group. Linear regression analysis was performed using BMDP Statistical Software Inc. (University of California Press, Berkeley, California, 1991).

Results

Reliability of measurements

The analytical variation in the compatible solute determinations (assessed by replicate analyses of a pig medulla homogenate) ranged from a coefficient of variation (relative SD) of 3% for GPC to 15% for glycine betaine.

Recovery of methylamines and polyols were 75% to 95% over the range of concentrations studied.

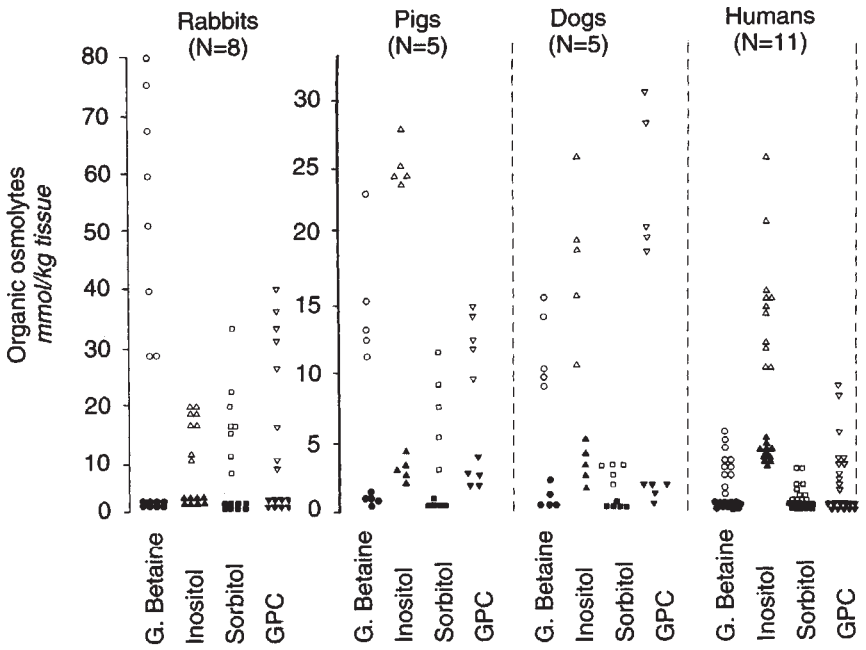

Fig. 1. *Organic osmolyte contents of mammalian kidney tissues.* Cortex and medulla samples assayed for glycine betaine (g.betaine), myo-inositol (inositol), sorbitol and glycerophosphorylcholine (GPC). Closed markers represent cortex, and open markers medulla. All results are expressed as mmol/kg wet weight.

Table 1. Compatible solutes in NZW rabbit tissues

Tissue (N = 5)	G. betaine	Inositol	Sorbitol	GPC
Cortex	0.36 (0.02)	2.4 (0.6)	0.07 (0.01)	1.7 (0.6)
Muscle	<2[a]	0.48 (0.06)	0.40 (0.40)	0.41 (0.56)
Bladder	0.21 (0.30)	3.3 (2.5)	0.03 (0.05)	0.9 (1.2)
Ureter	0.7 (0.1)	2.2 (2.3)	not detected	2.7 (2.2)

All results are mean (SEM), mmol/kg wet weight.
[a] The glycine betaine in muscle extracts was obscured, but 2 mmol/kg wet weight would have been detected

Table 2. Solute concentrations in human and rabbit inner medulla

	Human (N = 11)	Rabbit (N = 8)
Glycine betaine	2.7 (0.4)	55.5 (7.1)
Myo-inositol	15.1 (1.3)	16.4 (1.2)
Sorbitol	1.4 (0.3)	18.5 (2.4)
GPC	3.2 (0.5)	23.4 (3.3)
Sodium	97 (3.5)	112 (8.8)
Urea	39 (4.7)	137 (21)

All results are mmol/kg wet weight, mean (SEM).

Tissue levels

The early work on tissues from the dogs and pigs used perchloric acid as the extraction solvent. Later studies on rabbit and human kidneys used a double extraction with acetonitrile and methanol mixtures. Preliminary analysis of tissue samples with the different extraction procedures showed identical results for organic osmolyte levels for both methods.

For all mammalian species tested, including humans, the concentrations of the organic osmolytes glycine betaine, sorbitol, GPC and myo-inositol were much higher in the inner medulla than in the renal cortex (Fig. 1). This was true in each individual kidney; the kidneys with relatively high levels of an osmolyte in the cortex had correspondingly high levels in the medulla, and conversely for low medullary levels. Other tissues studied (rabbit muscle, bladder and ureter) contained levels similar to or less than the renal cortex (Table 1), but with more variability between individual animals. The largest proportionate differences were for sorbitol and glycine betaine, which were present at very low levels (less than 0.5 mmol/kg wet wt) in tissues other than the renal medulla. Myo-inositol and GPC were found in concentrations similar to the renal cortex in a wide variety of tissues.

The human tissues conformed to the pattern found in other mammals, however only myo-inositol reached concentrations in the inner medulla comparable to the animal kidneys. The low human inner medulla osmolyte levels were matched by low sodium and urea values, whereas the higher osmolyte levels in the rabbit occurred with high sodium and urea levels (Table 2). Linear regression analysis of the human and rabbit inner medulla data with urea, Na^+ or their sum as the independent variables showed there were a number of significant correlations (Table 3). In the humans the strongest correlation was between total methylamine and urea concentrations ($r = 0.79$, $P = 0.003$, Fig. 2).

More detailed dissection and analysis of seven human kidneys confirmed the higher concentrations of organic osmolytes in the inner medulla with a step-wise increase in glycine betaine, sorbitol and glycerophosphorylcholine along the cortico-papillary axis. Myo-inositol was found in highest concentration at the papillary base with high levels found also in the outer medulla and papillary tip (Fig. 3).

Unlike the animal kidneys, the human kidneys were subjected to a relatively long period of warm ischemia between nephrectomy and freezing. To exclude an artifactual lowering of osmolyte concentrations because of this sampling, extraction and storage variations were investigated by taking both kidneys

Table 3. Linear regression correlations for osmolytes in human and rabbit inner medulla

	Urea	Sodium	Urea + sodium
		r	
Rabbit (N = 8)			
G. betaine	0.89[c]	0.93[c]	0.92[c]
Myo-inositol	0.70[a]	0.78[a]	0.74[a]
Sorbitol	0.96[c]	0.76[a]	0.92[c]
GPC	0.97[c]	0.93[c]	0.98[c]
G. betaine + GPC	0.95[c]	0.96[c]	0.97[c]
Total osmolytes	0.97[c]	0.95[c]	0.99[c]
Human (N = 11)			
GPC	0.74[b]	NS	NS
G. betaine + GPC	0.79[b]	NS	NS
Total osmolytes	0.59[a]	NS	NS

[a] $P < 0.05$
[b] $P < 0.01$
[c] $P < 0.001$
Regression results in human kidneys for g. betaine, myo-inositol and sorbitol were not significant (NS).

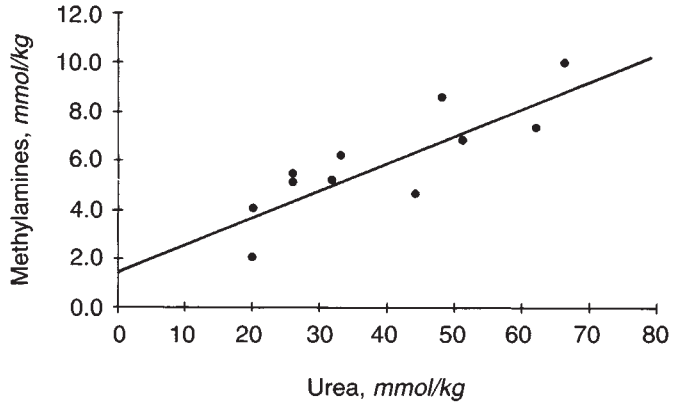

Fig. 2. *Relation between total methylamines (GPC + glycine betaine) and urea in human inner medulla* (N = *11*). All results are expressed as mmol/kg wet weight. r = 0.79; P = 0.003.

from each of five dogs and storing one at room temperature for two hours before analysis. Separate dissections and extractions of renal inner medullary tissue were made for each pair, and the reproducibility of the results confirmed that these variations were much less than the physiological variations of interest (Table 4).

Discussion

The organic osmolytes glycine betaine, myo-inositol, sorbitol and glycerophosphorylcholine have been found in high concentrations in the renal inner medulla of several species, including the dog, rat, rabbit and xeric and mesic rodents [6–11]. In the mammalian kidney the cells of the inner medulla are normally exposed to high concentrations of urea and extracellular sodium chloride [1]. However, in the rat inner medulla the sum of the intracellular concentrations of sodium, potassium and chloride was considerably lower than the corresponding sum of the extracellular concentrations [2]. It has been proposed that the renal medullary cells adapt to their environment by accumulating these organic osmolytes to fill this "osmotic gap" [5]. Glycine betaine and glycerophosphorylcholine may also counteract the effects of high urea concentrations on protein structure and function [3, 4].

Animal studies have demonstrated that the osmolytes respond to changes in osmotic pressure in the inner medulla. In rabbits and rats the levels of the four osmolytes were found to be higher during antidiuresis, and to decrease acutely in response to diuretic stimuli [11]. Cell culture experiments, with renal inner medullary cell lines, have shown accumulation of these same osmolytes in response to culture medium made hyperosmotic with sodium chloride or urea [16–19]. A decrease in culture medium osmolality resulted in a rapid efflux of osmolytes from the cells into the medium [19–22]. It seems likely that these same organic osmolytes will have a similar role in the human kidney, however, no previous studies have examined this theory.

The osmolyte levels in the kidneys we analyzed showed the same pattern in all species, including humans. There was a relatively high concentration in the renal inner medulla, but not in the renal cortex or other tissues. The more detailed dissections of the human kidneys confirmed that an increasing cortico-papillary gradient for sodium and urea was present, and in concert with this, increasing levels of all the osmolytes except myo-inositol, whose maximum levels occurred at the papillary base. This pattern has been reported in several animal species and suggests that myo-inositol has additional functions to its role as an osmolyte [8, 14].

Although a general mammalian pattern for the intra-renal distribution of osmolytes was apparent, there was a wide variation in osmolyte levels both within and between species. The concentrations of the four osmolytes in the human kidneys were considerably less than the animal kidneys. Without controlled conditions, including diet and hydration state, it is not possible to make direct comparisons. Several factors could have altered osmolyte concentrations in the human kidneys. These include diuresis due to administration of intravenous fluids before and during the operation, the effects of drugs used for inducing and maintaining anesthesia, and the relatively poor concentrating ability of the human kidney in this study's elderly population. The human kidneys were also subjected to a period of warm ischemia between the ligation of the vascular pedicle and freezing. Our data on the paired dog kidney experiments suggest that this additional insult had little effect on inner medulla osmolyte levels, making it an unlikely cause of the low osmolyte levels found in the human kidneys.

The low inner medullary concentrations of sodium and urea in the human kidneys were strong presumptive evidence for a relatively low osmotic pressure in the human inner medulla. It is therefore not surprising that the osmolyte levels we found in the human inner medulla were lower than the levels found in the animal kidneys. In contrast, the higher osmolyte levels in the rabbit occurred with higher sodium and, particularly, urea levels. The relatively high levels of myo-inositol and low levels of the other osmolytes found in the human inner medulla have been noted previously in medullary cell cultures in conditions of low sodium chloride and in diuretic kidneys [23], circumstances similar to the human kidneys we analyzed.

If the osmolytes balance the excess osmotic pressure due to sodium chloride, and the methylamines protect cells from urea toxicity, the osmolytes should change *parri passu* with sodium chloride and urea. In the rabbits there were strong correlations

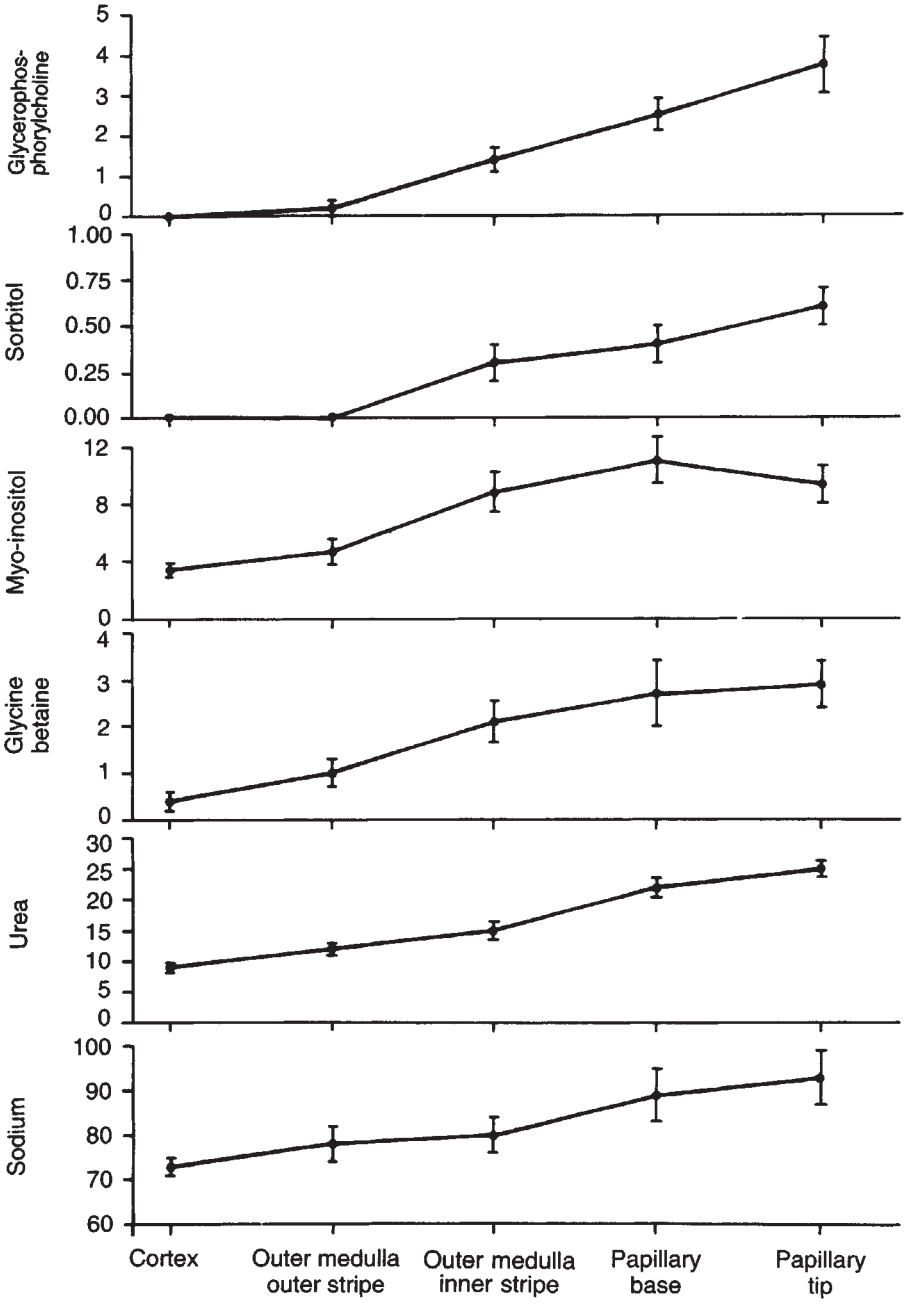

Fig. 3. *Sodium and organic osmolyte levels (mmol/kg wet weight) along cortico-papillary axis in human kidneys.* Results are expressed as mean (SEM), $N = 7$.

between the osmolytes and sodium chloride and urea. However, in the humans, correlations were found only with urea as the independent variable. The strong correlation between urea and methylamine (glycine betaine + glycerophosphorylcholine) concentrations we demonstrated in the human and rabbit inner medulla supports the counteracting osmolytes hypothesis between urea and methylamines [3, 4]. On the assumption that the two methylamines are largely intracellular while urea freely enters cells, the ratio of urea:methylamines in the human inner medulla was close to 2:1, the value previously reported as optimal for this protective effect. The failure to demonstrate

Table 4. Inner medulla osmolyte concentrations in paired dog kidneys

	Glycine betaine	Myo-inositol	Sorbitol	GPC
Rapid frozen				
Mean	11.0	16.5	1.6	21.1
SD	3.0	5.7	0.9	7.5
SEM	1.4	2.6	0.4	3.4
Slow frozen				
Mean	12.7	15.2	1.7	19.9
SD	3.4	5.7	0.8	7.4
SEM	1.5	2.6	0.4	3.3

All results are mmol/kg wet weight.

significant correlations between sodium and osmolytes in the human kidneys may have been due to the labile osmotic conditions under which the kidneys were obtained. Animal studies have shown a disparate recovery of osmolyte, sodium and urea gradients in response to rapid alterations in hydration state [11]. In contrast, the rabbits, which were in metabolic cages and on controlled diets, did not have sudden alterations in hydration conditions prior to sacrifice.

The present study demonstrates that the same four organic osmolytes found in other mammalian kidneys are also present in the human kidney. The distribution and levels of the osmolytes are consistent with the proposed functions of filling the "osmotic gap" and counteracting urea toxicity. It is very likely that the osmolyte system is essential for normal function of the human inner medulla. Animal models can be used to further investigate the organic osmolyte system in the mammalian kidney and its response to various physiological and pathological conditions.

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References

1. JAMISON RL, KRIZ W: *Urinary Concentrating Mechanism: Structure and Function*, New York, Oxford, Oxford University Press, 1982, pp. 79–90
2. BECK F, DORGE A, RICK R, THURAU K: Intra- and extracellular element concentrations of rat renal papilla in antidiuresis. *Kidney Int* 25:397–403, 1984
3. YANCEY PH, CLARK ME, HAND SC, BOWLUS RD, SOMERO GN: Living with water stress: Evolution of osmolyte systems. *Science* 217:1214–1222, 1982
4. SOMERO GN: Protons, osmolytes, and fitness of internal milieu for protein function. *Am J Physiol* 251:R197–R213, 1986
5. BALABAN RS, BURG MB: Osmotically active organic solutes in the renal inner medulla. *Kidney Int* 31:562–564, 1987
6. MARTIN J, FINKELSTEIN J: Enzymatic determination of betaine in rat tissues. *Anal Biochem* 111:72–76, 1981
7. COHEN MAH, HRUSKA KA, DAUGHADAY WH: Free myo-inositol in canine kidneys: Selective concentration in renal medulla. *Proc Soc Exp Biol Med* 169:380–385, 1982
8. BAGNASCO S, BALABAN RS, FALES H, YANG Y-M, BURG M: Predominant osmotically active solutes in rat and rabbit renal medullas. *J Biol Chem* 261:5872–5877, 1986
9. GULLANS SR, BLUMENFELD JD, BALSCHI JA, KALETA M, BRENNER RM, HEILIG CW, HEBERT SC: Accumulation of major organic osmolytes in rat inner medulla in dehydration. *Am J Physiol* 255:F626–F634, 1988
10. YANCEY PH: Osmotic effectors in kidneys of xeric and mesic rodents: Corticomedullary distributions and changes with water availability. *J Comp Physiol* 158(3):369–380, 1988
11. WOLFF SD, STANTON TS, JAMES SL, BALABAN RS: Acute regulation of the predominant organic solutes of the rabbit renal inner medulla. *Am J Physiol* 257:F676–F681, 1989
12. *Geigy Scientific Tables*. (8th ed) Edited by LENTNER C, New Jersey, Ciba-Geigy, 1981, vol 1, p. 84
13. CHAMBERS ST, KUNIN CM: Isolation of glycine betaine and proline betaine from human urine. *J Clin Invest* 79:731–737, 1987
14. YANCEY PH, BURG MB: Distribution of major organic osmolytes in rabbit kidneys in diuresis and antidiuresis. *Am J Physiol* 257:F602–F607, 1989
15. LEVER M, BASON L, LEAVER C, HAYMAN CM, CHAMBERS ST: Same-day batch measurement of glycine betaine, carnitine and other betaines in biological material. *J Anal Chem Anal Biochem* 205:14–21, 1992
16. NAKANISHI T, BALABAN RS, BURG MB: Survey of osmolytes in renal cell lines. *Am J Physiol* 255:C181–C191, 1988
17. NAKANISHI T, TURNER J, BURG MB: Osmoregulation of betaine transport in mammalian renal medullary cells. *Am J Physiol* 258: F1061–F1067, 1990
18. MORIYAMA T, GARCIA-PEREZ A, BURG MB: High urea induces accumulation of different organic osmolytes than does high NaCl in renal medullary cells. (abstract) *Kidney Int* 37:586, 1990
19. YAMAUCHI A, KWON HM, UCHIDA S, PRESTON AS, HANDLER JS: Myo-inositol and betaine transporters regulated by tonicity are basolateral in MDCK cells. *Am J Physiol* 261:F197–F202, 1991
20. BAGNASCO SM, MURPHY HR, BEDFORD JJ, BURG MB: Osmoregulation by slow changes in aldose reductase and rapid changes in sorbitol flux. *Am J Physiol* 254:C788–C792, 1988
21. NAKANISHI T, BURG MB: Osmoregulation of glycerophosphorylcholine content of mammalian renal cells. *Am J Physiol* 257:C795–C801, 1989
22. NAKANISHI T, BURG MB: Osmoregulatory fluxes of myo-inositol and betaine in renal cells. *Am J Physiol* 257:C964–C970, 1989
23. MORIYAMA T, GARCIA-PEREZ A, BURG MB: Factors affecting the ratio of different organic osmolytes in renal medullary cells. *Am J Physiol* 259:F847–F858, 1990